Bioremediation of Hydrocarbon and Some Heavy Metal Polluted Wastewater Effluent of a Typical Refinery

S. Abdulsalam, A. D. I. Suleiman, N. M. Musa, M. Yusuf

Abstract**—**Environment free of pollutants should be the concern of every individual but with industrialization and urbanization it is difficult to achieve. In view of achieving a pollution limited environment at low cost, a study was conducted on the use of bioremediation technology to remediate hydrocarbons and three heavy metals namely; copper (Cu), zinc (Zn) and iron (Fe) from a typical petroleum refinery wastewater in a closed system. Physicochemical and microbiological characteristics on the wastewater sample revealed that it was polluted with the aforementioned pollutants. Isolation and identification of microorganisms present in the wastewater sample revealed the presence of *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus* and *Staphylococcus epidermidis*. Bioremediation experiments carried out on five batch reactors with different compositions but at same environmental conditions revealed that treatment T5 (boosted with the association of *Bacillus subtilis, Micrococcus luteus*) gave the best result in terms of oil and grease content removal (i.e. 67% in 63 days). In addition, these microorganisms were able of reducing the concentrations of heavy metals in the sample. Treatments T5, T3 (boosted with *Bacillus subtilis* only) and T4 (boosted with *Micrococcus luteus* only) gave optimum percentage uptakes of 65, 75 and 25 for Cu, Zn and Fe respectively.

Keywords**—**Boosted, bioremediation, closed system, aeration, uptake, wastewater.

I. Introduction

PETROLEUM refinery utilizes large quantity of water for various operation such as desalting, distillation, thermal cracking, catalytic and other treating processes to produce useful and valuable products like liquefied petroleum gas (LPG), gasoline, jet fuel, diesel, asphalt and petroleum feedstock [1], [2]. In the same vein, refinery processes generate large volume of wastewater; the volume of wastewater generate was estimated to be between 0.4–1.6 times the volume of crude oil processed [3].

The indiscriminate ways most industries discharge their waste products into the environment is of serious environmental concern because of the hazard associated with them. These wastes can either be in solid, liquid or gaseous states. In a typical petroleum refinery these three classes of wastes aforementioned are present but attention was focused on the liquid waste (refinery wastewater) in this study.

Refinery wastewater may contain constituents such as heavy metals, hydrocarbons and other harmful constituents at concentrations above the threshold limits in the environment when discharged untreated. These pollutants got into the water body through run off water or through leaching into groundwater thereby contaminating various sources of potable water which in turn pose great danger to aquatic lives and human.

Bioremediation is a technique that uses microorganisms to accelerate degradation and/or removal of contaminants from the environment. The uses of microbial metabolic ability for degradation/removal of environmental pollutants are safe and economical compared to the physicochemical technologies. Although highly diverse and specialized microbial communities present in the environment do efficiently remove many pollutants, this process is usually quite slow, which leads to a tendency for pollutants to accumulate in the environment, which can potentially be hazardous. This is especially true for heavy metals.

Heavy metal contamination is one of the most significant environmental issues, since metals are highly toxic to biota, as they decrease metabolic activity and diversity, and they affect the qualitative and quantitative structure of microbial communities. For treating heavy metal contaminated tailings and soils, bioremediation is still the most cost-effective method, although various heavy metals are beyond the bioaccumulation capabilities of microorganisms. Perhaps, because of the toxicity of these compounds, microorganisms have not evolved appropriate pathways to bioaccumulate them because populations of microorganisms responsible for this bioaccumulation are not large or active enough to remove these compounds completely, or complex mixtures of pollutants resist removal by existing pathways. The pathway used to accumulate these compounds is adsorption, where metals are taken up by microbial cells (biosorption).

Biosorption mechanisms are numerous and are not yet fully understood. However, biosorption capacity often varies with test conditions, such as initial metal concentration, solution pH, contact time, biomass dosage, processing method, and so on. Accordingly, populations of microorganisms that are able to promote metal adsorption and accumulate them are not large or active enough to support these compounds by existing

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pathways. Furthermore, there are several strategies that optimize the bioremediation process of pollutants. One approach to enhance populations of microorganisms capable of pollutant removal is the addition of exogenous microorganisms in order to expand indigenous populations.

Heavy metals are considered to be chemical elements with an atomic mass greater than 22 and a density greater than 5g/mL. This definition includes 69 elements, of which 16 are synthetic. Some of these elements are extremely toxic to human beings, even at very low concentrations [4], [5]. The main heavy metals associated with environmental contamination, and which offer potential danger to the ecosystem, are copper (Cu), zinc (Zn), silver (Ag), lead (Pb), mercury (Hg), arsenic (As), cadmium (Cd), chromium (Cr), strontium (Sr), cesium (Cs), cobalt (Co), nickel (Ni), thallium (Tl), tin (Sn) and vanadium (V) [5].

Among the different contaminants, heavy metals have received special attention due to their strength and persistence in accumulating in ecosystems, where they cause damage by moving up the food chain to finally accrue in human beings [6]-[8].

Due to the health hazard associated with the petroleum refinery wastewater and high cost of treatment using physicochemical technologies, this study centered on the bioremediation of hydrocarbon and some heavy metal contents of a typical petroleum refinery wastewater. The objectives were to; determine the physicochemical and microbiological characteristics of the contaminated wastewater, develop an effective treatment (s) for detoxification of oil & grease and the heavy metal contents, and determine the microbial types capable of remediating these contaminants.

II. Materials and Methods

Wastewater sample was collected in two sterile plastic containers of 50 L capacity each from a typical petroleum refinery in Kaduna State-Nigeria. This sample was preserved at 4ºC in a deep freezer. The physicochemical and microbiological characteristics of this sample were investigated using established standard methods.

The physicochemical parameters of the wastewater samples were carried out using the Federal Water Pollution Control Act (FWPCA) methods for chemical analysis of water and wastewater and photometric method for analysis of sewage and industrial wastewater. Spectrophotometric methods were employed for heavy metal determination (USEPA) and chemical oxygen demand (COD) using DR/2000 spectrophotometer. In addition, the oil and grease content of each sample was determined by employing the reflux method using the DR/2000 Spectrophotometer (USEPA). Some of the physicochemical parameters carried out were; pH, conductivity, turbidity, total dissolved solids (TDS), total suspended solids (TSS), total nitrogen (N), total phosphorus (P), biological oxygen demand (BOD), chemical oxygen demand (COD) and oil and grease content. Heavy metals investigated were; copper (Cu), zinc (Zn) and iron (Fe). The microbiological characteristics carried out on the wastewater samples were the total heterotrophic bacteria count using the serial dilution plate counting technique, hydrocarbon degrading bacterial count using the Bushnell Hass medium, and isolation and identification of bacteria present in the wastewater sample employing the gram staining, morphological and biochemical reactions. The isolates present were identified using Beryer's manual of systematic bacteriology.

A. Experimental Design

The various treatments used in this study are presented in Table I.

TABLE I
Composition of Various Treatments

Treatment	Composition
T1	Wastewater
T2	Wastewater + Aeration @ 72 L/h
T3	Wastewater + Isolate 1 + Aeration @ 72 L/h
T4	Wastewater + Isolate 2 + Aeration @ 72 L/h
T5	Wastewater + Isolates 1 & 2 + Aeration @ 72 L/h

Isolate 1: *Bacillus subtilis*; Isolate 2: *Micrococcus luteus*

B. Bioremediation Experiment

Treatments 1 to 5 presented in Table I were set up as illustrated in Fig. 1. Air was compressed on continuous basis at a flow rate of 72 L/h to all the treatments except treatment 1 (T1) for sixty three days. Each treatment was placed in a different set up and all set ups were operated at the same environmental conditions. Samples were taken from each of the five set ups for analyses on weekly basis. Parameters monitored include; oil and grease content (O & G), heavy metal concentrations, and other physicochemical parameters as indicated in Table II.

III. Results and Discussion

A. Physicochemical Parameters

The physicochemical parameters of the wastewater samples were taken before and after treatment as presented in Table II. Of primary important at this stage was the composition of the inorganics (i.e. carbon (C), nitrogen (N) and phosphorus (P)). From the result of the elemental composition as presented in Table II, the carbon/nitrogen molar ratio was 1:2 which was sufficient for effective bioremediation to take place. This molar ratio was far above the carbon/nitrogen molar ratio of 1:10 reported by most literatures for effective bioremediation to occur [9], [10]. From the aforementioned, addition of inorganic nutrients was not required for the treatment. In addition, when the physicochemical parameters taken before treatment of the wastewater sample were compared with the Nigerian Standard for Drinking Water Quality (NSDWQ), it revealed that the wastewater in question was highly contaminated judging from the values of turbidity (47 NTU), carbon (49.25 mg/L), O&G content (21 mg/L), Cu^{2+} (3.5 mg/L) and Fe^{2+} (0.93 mg/L) which were above maximum limits set by NSDWQ (Table II). Hence, the wastewater sample required intervention for public health and environmental safety. However, since the scope for this study was limited to remediation of hydrocarbon and heavy metals, holistic treatment of wastewater was not carried out in this study.

B. Microbiological Analysis

The total heterotrophic bacteria and hydrocarbon degrading bacteria counts were carried out on the wastewater sample, results of these two important parameters revealed that sufficient number of bacteria and hydrocarbon degrading ones were present for effective bioremediation to take place [11]. Isolation and identification of bacteria strains in the test samples revealed the presence of *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus* and *Staphylococcus epidermidis*. These four strains are hydrocarbon degrading [12]-[14] but due to versatility and wide range of applications of *Bacillus subtilis* and *Micrococcus luteus* in bioremediation studies [10], [15], these two were employed to boost the activities of the indigenous microorganisms in various ways as indicated in Table I. This is in order to overcome the resistance of heavy metals by microorganisms [16].

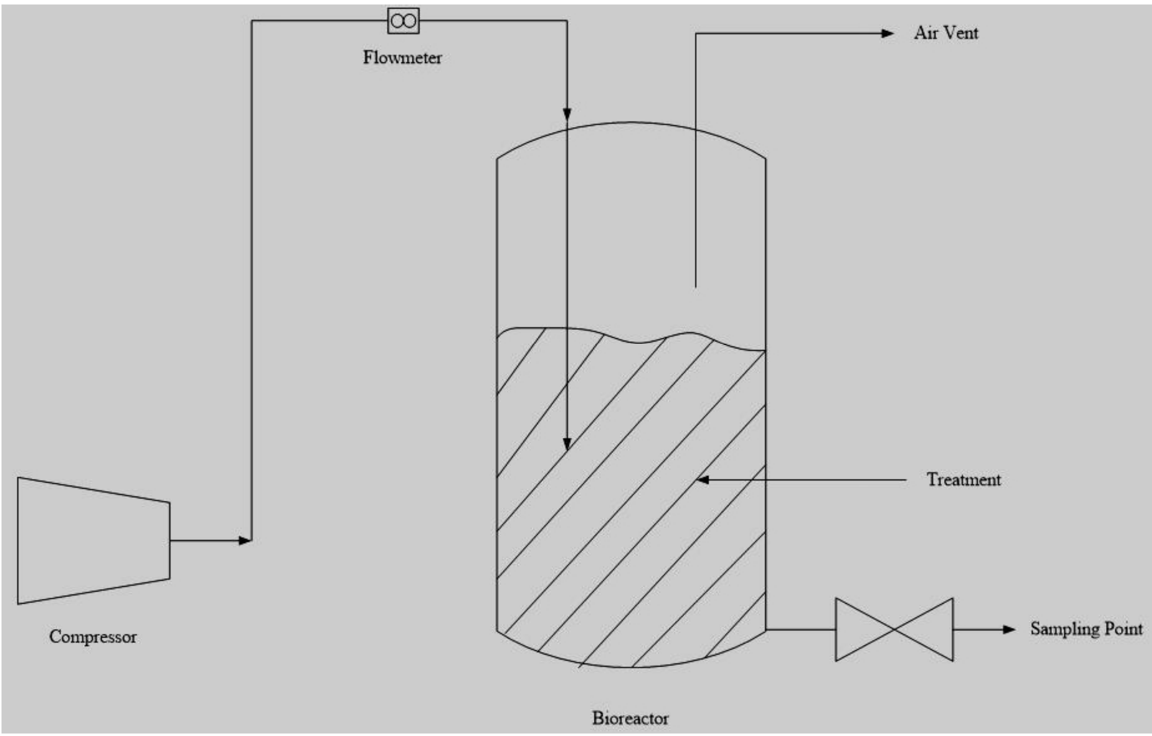

Fig. 1 Schematic representation of a process for bioremediation of petroleum refinery wastewater

C. Bioremediation of Samples Oil and Grease Content

Fig. 2 presents the variation of oil and grease contents with time for the five treatments considered in this study. It could be seen that in all treatments, O&G contents decreased with time which was in line with the general degradation principle [17].

Considering treatments 1 and 2 (T1 and T2), the effect of aeration was clearly seen judging from the percentage removal of O&G contents. The percent removal was 15 for T1 and 22 for T2 (Table II). This showed that aeration at 72 L/h had positive impact on the biodegradation of O&G content of the petroleum refinery wastewater used. In the same vein, T1 with no alteration or stimulation recorded appreciable level of degradation at a slower rate compared to T2. This observation was also in line with the literature [12].

Addition of individual or consortium of isolates as shown in treatments 3-5 (T3-T5) showed an improvement over T2. The percentage O&G content removals were 50, 43 and 67 for T3, T4 and T5 respectively. These results showed that *Bacillus subtilis* was a better hydrocarbon degrader than *Micrococcus luteus* under the experimental conditions used and the association of these two isolates had better effect on hydrocarbon removal than the individual isolates. These observations are also in agreement with the literature [12], [13].

Turbidity and TDS followed similar trend as the O&G content removal as would be expected. The higher the removal of O&G content, the higher the values of turbidity and TDS which is proportional to the growth of microorganisms. On the other hand, BOD decreased as the O&G content removal increased, and the COD followed similar trend as BOD except for T5.

For all the treatments (T1-T5), the pH values fell within the acceptable limit for effective bioremediation [10], [18], [19] and fell within the safe limit by standard. In addition, the conductivity for all the treatments was within the acceptable limit except for T5.

TABLE II. A
IMPORTANT PARAMETERS OF THE INDUSTRIAL WASTEWATER AFTER BIOREMEDIATION

Parameter	Before	*Standard (max. allowable)
Physicochemical		
pH	6.55	**6.50-8.50**
Conductivity (μs.cm^{-1})	450.00	**1000**
Turbidity (NTU)	47.00	**5.00**
TDS (mgl^{-1})	224.00	**500**
TSS (mgl^{-1})		47.00
Organics		
BOD (mgl^{-1})	155.00	
COD (mgl^{-1})	285.00	
O&G (mgl^{-1})	21.00	
Heavy Metals		
Cu^{2+} (mgl^{-1})	3.50	**1.00**
Zn^{2+} (mgl^{-1})	0.08	**3.00**
Fe^{2+} (mgl^{-1})	0.93	**0.30**
Inorganics		
Phosphate (mgl^{-1})		7.36
Total Nitrogen (N), (mgl^{-1})	28.68	
Total Carbon (C), (mgl^{-1})	49.25	**5.00**

Microbiological Analysis Before Treatment

THBC (CFU/ml): @ dilution factors of 10^{-4}, 10^{-6} & 10^{-8} were $1.5*10^7$, $1.06*10^8$ & $9.4*10^{10}$ respectively

HDBC (CFU/ml): @ dilution factors of 10^{-4}, 10^{-6} & 10^{-8} were $6.0*10^6$, $4.5*10^7$ & $3.0*10^{10}$ respectively.

Isolates identified *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus* and *Staphylococcus epidermidis*

TABLE II. B
IMPORTANT PARAMETERS OF THE INDUSTRIAL WASTEWATER AFTER BIOREMEDIATION

Parameter	T1	T2	T3	T4	T5
Physicochemical					
pH	8.30	7.40	7.20	7.65	7.67
Conductivity (μs.cm^{-1})	479.00	504.00	741.33	690.33	1366.67
Turbidity (NTU)	59.67	72.67	94.00	83.00	145.33
TDS (mgl^{-1})	238.67	261.33	381.33	355.00	678.00
TSS (mgl^{-1})	37.00	32.33	43.67	53.00	132.33
Organics					
BOD (mgl^{-1})	139.33	107.00	67.33	78.67	57.67
COD (mgl^{-1})	250.00	197.33	129.67	138.67	155.67
O&G (mgl^{-1})	17.80	16.40	10.50	12.00	7.00
Heavy Metals					
Cu^{2+} (mgl^{-1})	2.21	1.74	1.35	1.57	1.21
Zn^{2+} (mgl^{-1})	0.03	0.03	0.02	0.02	0.03
Fe^{2+} (mgl^{-1})	0.84	0.81	0.77	0.70	0.70

D. Variation of Heavy Metals with Time

Biodegradation potentials of some heavy metals (Cu, Zn & Fe) present in the wastewater sample were studied. Results obtained are presented in Figs. 3-5, from the profiles obtained; the three heavy metals were susceptible to biodegradation with optimum degradations of 65% by T5 (consortium of *Bacillus subtilis* and *Micrococcus luteus*), 75% by T3 (*Bacillus subtilis*) and 25% by T4 (*Micrococcus luteus*) for Cu, Zn and Fe respectively. Therefore, *Bacillus subtilis* and *Micrococcus luteus* have affinities to degrade Cu, Zn and Fe. Although, the residual final concentrations of Cu and Fe in this study were above the permissible limits by NSDWQ, running the experiments for longer period and/or varying some process conditions such as air flow rate, temperature, increase in population of exogenous bacteria or types and pH could lead to attaining the desired limits.

Generally, it was observed that the bioaccumulation rate of *Bacillus subtilis* was higher than that of *Micrococcus luteus* for Cu and Fe, and that of *Micrococcus luteus* higher than that of *Bacillus subtilis* for Zn.

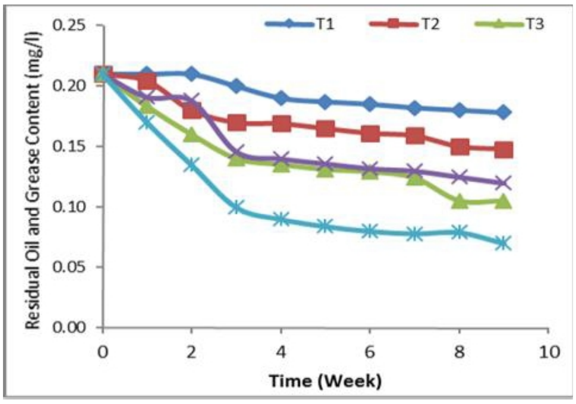

Fig. 2 Variation of Oil and Grease Content with Bioremediation Time

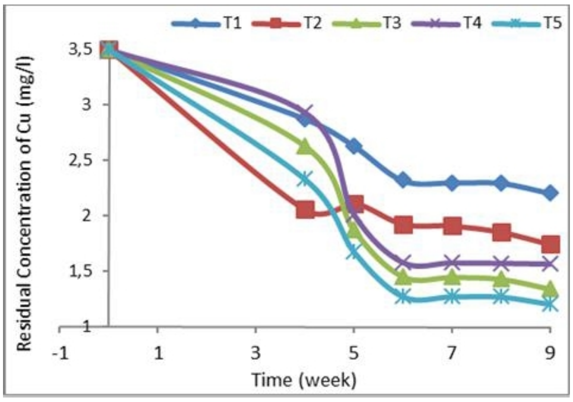

Fig. 3 Variation of Cu Concentration with Bioremediation Time

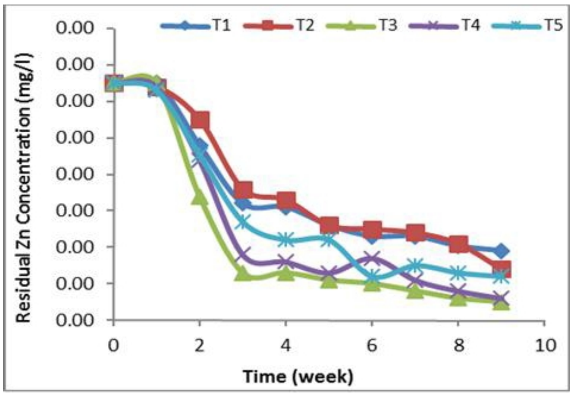

Fig. 4 Variation of Zn Concentration with Bioremediation Time

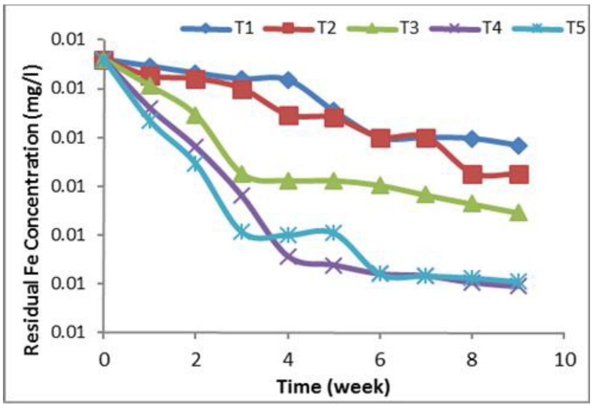

Fig. 5 Variation of Fe Concentration with Bioremediation Time

IV. Conclusion

Four hydrocarbon degrading bacteria were identified from the wastewater sample: *Bacillus subtilis, Micrococcus luteus, Staphylococcus aureus* and *Staphylococcus epidermidis*. Treatment T5 (boosted with the association of *Bacillus subtilis* & *Micrococcus luteus*) gave the best oil and grease content removal (i.e. 67% in 63 days) while T1 gave the least performance (15%). In addition, *Bacillus subtilis* and *Micrococcus luteus* were able to reduce the concentrations of heavy metals in the samples: Treatments T5, T3 (boosted with *Bacillus subtilis*) and T4 (boosted with *Micrococcus luteus*) gave optimum percentage metal uptakes of 65, 75 and 25 for Cu, Zn and Fe respectively.

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